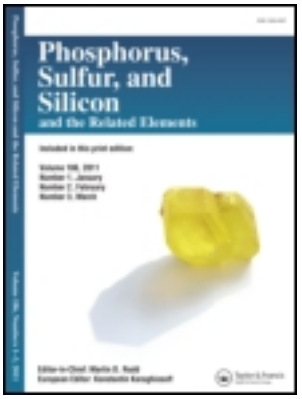

Phosphorus, Sulfur, and Silicon and the Related Elements

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Synthesis, Antibacterial, and Analgesic Activity of Novel 4-Hydroxy-3-(phenylthio)-2H-chromen-2-ones and 4-Hydroxy-3-[imidazol/tetrazolo-2-yl)thio]-2H-chromen-2-ones

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SYNTHESIS, ANTIBACTERIAL, AND ANALGESIC ACTIVITY OF NOVEL 4-HYDROXY-3-(PHENYLTHIO)-2*H*-CHROMEN-2-ONES AND 4-HYDROXY-3-[IMIDAZOL/TETRAZOLO-2-yl)THIO]-2*H*-CHROMEN-2-ONES

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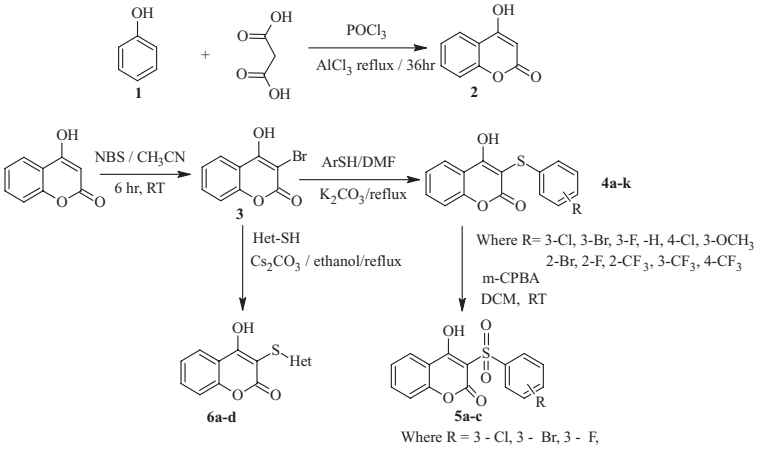
GRAPHICAL ABSTRACT



Abstract *A mild and efficient synthesis of new 4-hydroxy-3-(phenylthio)-2H-chromen-2-ones* ***4a–k*** *and 4-hydroxy-3-[imidazol/tetrazolo-2-yl)thio]-2H-chromen-2-ones* ***6a–d*** *is described. The compounds* ***4a–c*** *were further subjected to oxidation by m-CPBA to obtain 3-(phenylsulfonyl)-4-hydroxy-2H-chromen-2-ones* ***5a–c****. The structures of the resulted new compounds were established by IR, ^{1}H NMR, mass, and elemental analysis. All the synthesized compounds were subjected to antibacterial activity against the Gram positive bacteria*

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Staphylococcus aureus, Bacillus subtilis, *and* Streptococcus haemolytius *and the Gram negative bacteria* Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, *and* Klebsiella pneumonia. *The analgesic activity was carried out using Swiss albino male mice by abdominal concentration method.*

Supplemental materials are available for this article. Go to the publisher's online edition of Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.*

Keywords Analgesic activity; antibacterial activity; phenyl coumarinyl thioethers

INTRODUCTION

Coumarin and its derivatives represent one of the most active classes of compounds possessing a wide spectrum of biological activity.[1–9] Many of these compounds have proved to be active as antitumor,[1,2] antibacterial,[3,4] antifungal,[5–7] anticoagulant,[8] and anti-inflammatory[9] agents. In addition, these compounds are also used as additives to food and cosmetics,[10] and have dispersed fluorescent and laser properties.[11] Various 3-substituted coumarins exhibit antimicrobial activity.[12,13]

The phenyl coumarinyl ethers constitute a group of naturally occurring oxygen heterocyclic compounds, isolated principally in the *Thymeliaceae* family but also in the *Leguminceae* and *Rutaceae* families and various other plants.[14–16] The various 3-substituted 4-hydroxy-coumarins are active nonpeptide HIV-PR inhibitors,[17,18] which opens up a new therapeutic possibility. In connection with the preparation of bioactive coumarins, the structure-activity relationships of various 3-substituted-4-hydroxy coumarins have also been reported.[19] In addition, some coumarins are of interest because of their toxicity,[20] carcinogenity,[21] and photodynamic effects.[22] Coumarins also act as intermediates for the synthesis of furocoumarins, chromenes, coumarones, and 2-acylresorcinols.[23] Some of the naturally occurring coumarin ethers analogs are coumarin glycoside[24] and daphnoretin.[15]

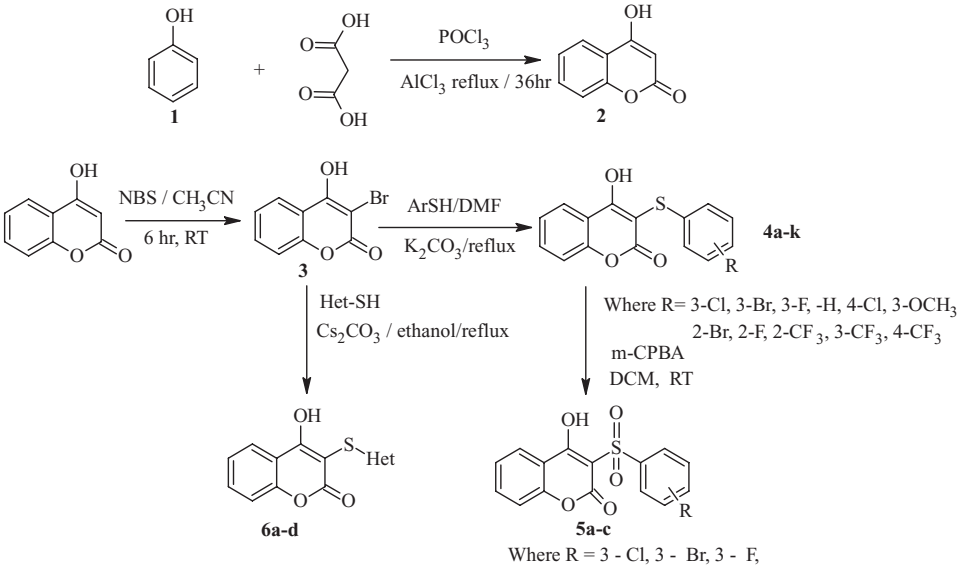
Thus, the influence of coumaryl ethers prompted us to synthesize various thiocoumaryl ethers by condensing various thiophenols and heterocyclic thiols with 3-bromo-4-hydroxy coumarin by developing a simple protocol, which is in continuation of our work on biological testing of natural products as well as various synthetic compounds.[25–31]

Hence, a series of new 4-hydroxy-3-(phenylthio)-2*H*-chromen-2-ones **4a–k** and 4-hydroxy-3-[imidazol/tetrazolo-2-yl)thio]-2*H*-chromen-2-ones **6a–d** was prepared in good yields by the condensation of 3-bromo-4-hydroxy-coumarin **2** with various thiophenols in the presence of K_2CO_3/DMF and Cs_2CO_3/EtOH. The compounds **4a–c** were further subjected to oxidation promoted by m-CPBA to obtain 3-(phenyl sulfonyl)-4-hydroxy-2*H*-chromen-2-ones **5a–c**. The structures of the resulted new compounds were established by IR, ^{1}H NMR, mass, and elemental analysis. All the synthesized compounds were subjected to the evaluation of their antibacterial activity against the Gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus haemolytius* and Gram negative bacteria *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*.

RESULTS AND DISCUSSION

The generation of the required substrate 3-bromo-4-hydroxy-coumarin **3** is readily achieved in 70–85% yield by the reaction of 4-hydroxycoumarin with NBS in acetonitrile at 25–27°C. The ^{1}H NMR of compound **3** exhibited a double doublet at 7.37–7.43 δ that corresponds to two protons, another triplet at 7.66–7.69 δ for one proton and a doublet at

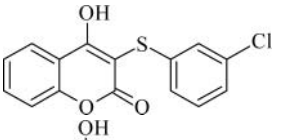
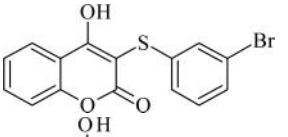
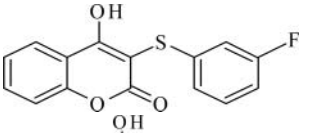
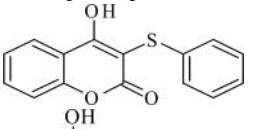
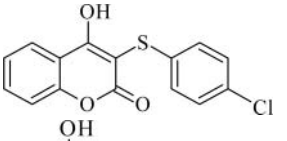
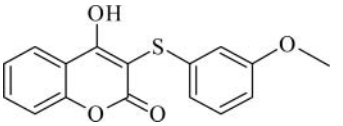
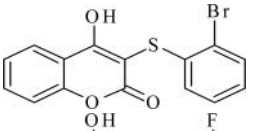
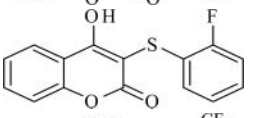
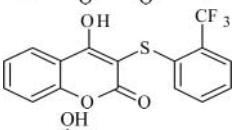
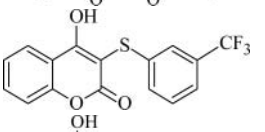
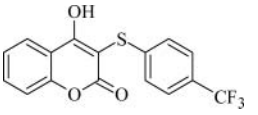
7.96–7.98 δ integrated for one proton confirms the structure of compound **3**. Further LC-MS analysis of compound 3 gave a molecular ion peak at 242 (M +1) to ascertain the structure of compound **3**. Thus, coumarin **3** was first utilized to couple with various thiophenols in the presence of base K_2CO_3 in DMF as solvent to obtain the corresponding 4-hydroxy-3-(phenylthio)-2*H*-chromen-2-ones **4a–k** in good yield (Table 1, Scheme 1) in which the 4-hydroxy group is necessary as much for its bonding to the HIV-PR for its inhibitory potency.[32] Besides their antithrombotic activity and analgesic activity, the 3-substituted 4-hydroxycoumarins also present interesting free-radical scavenging properties.[33] Hence the above biological information regarding 4-hydroxycoumarin derivatives prompted us to screen for their antibacterial and analgesic activities.



Scheme 1

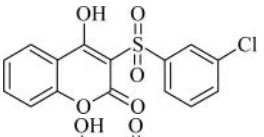
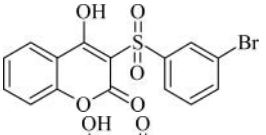
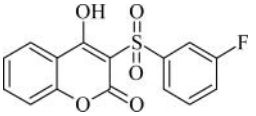
Further thiadiazole, triazole, tetrazole,[34] and other heterocyclic derivatives of 3-substituted coumarins[35,36] are well known for their biological activities such as antimicrobial,[37] anti-inflammatory,[38] and anticancer.[39] In view of the biological importance of the above-mentioned heterocyclic compounds, we thought it worthwhile to develop a new method for the rapid synthesis of such heterocyclic rings connected to coumarin through a sulfur bridge. Thus 2-mercaptoimidazole, 1-*N*-methyl-2-mercaptoimidazole, 5-mercapto-tetrazole, 1-*N*-methyl-5-mercapto-tetrazole are commercially available and were used for the reaction without further purification (Scheme 1). So the various 4-hydroxy-3-[imidazol/tetrazolo-2-yl)thio]-2*H*-chromen-2-ones **6a–d** were prepared in good yields by the condensation of 3-bromo-4-hydroxy-coumarin **2** with various 2-mercaptoimidazole, 1-*N*-methyl-2-mercaptoimidazole, 5-mercapto-tetrazole, 1-*N*-methyl-5-mercapto-tetrazole, in the presence of base Cs_2CO_3 in EtOH as solvent at reflux temperature. In this case, the base such as K_2CO_3 did not give the expected 4-hydroxy-3-[imidazol/tetrazolo-2-yl)thio]-2*H*-chromen-2-ones **6a–d** in good yield, hence Cs_2CO_3 was used instead.

Table 1 Reaction time, yield, and melting points of newly synthesized compounds **4a–k**

Product	Structure[a]	Yield (%)[b]	Mp °C	Time (h)
4a		79	159–160	5
4b		86	220–222	5
4c		58	166–167	5
4d		53	200–202	5
4e		73	197–198	5
4f		60	176–177	5
4g		83	210–213	5
4h		86	220–222	5
4i		82	227–228	5
4j		85	217–218	5
4k		71	136–137	5

[a]Reaction was carried out at reflux temperature in DMF.
[b]Isolated yields.

Table 2 Reaction time, yield, and melting points of newly synthesized compounds **5a–c**

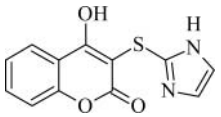
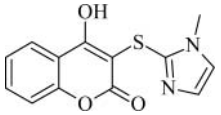
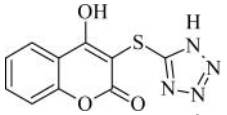
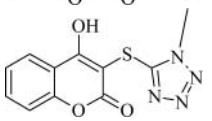
Product	Structure[a]	Yield (%)[b]	Mp (°C)	Time (h)
5a		82	170–172	7
5b		75	202–204	8
5c		80	130–133	7

[a]Reaction was carried out at room temperature in DCM.
[b]Isolated yields.

To make variations in the structure, the compounds **4a–c** were oxidized with m-chloroperbenzoic acid in dichloromethane at 25–27°C to afford the corresponding 3-(phenyl sulfonyl)-4-hydroxy-2*H*-chromen-2-one **5a-c** in the yield of 60–75% (Table 2, Scheme 1).

In a typical experiment, 2-mercaptoimidazole was condensed with 3-bromo-4-hydroxycoumarin in the presence of Cs_2CO_3 in ethanol to obtain the corresponding 4-hydroxy-3-[(1*H*-imidazol-2-yl)thio]-2*H*-chromen-2-ones, 4-hydroxy-3-[(1-methyl-1*H*-imidazol-2-yl)thio]-2*H*-chromen-2-ones **6a** and **6b** and 4-hydroxy-3-[(1*H*-tetrazol-5-yl)thio]-2*H*-chromen-2-ones,4-hydroxy-3-[(1-methyl-1*H*-tetrazol-5-yl)thio]-2*H* chromen-2-ones **6c** and **6d**, respectively (Table 3).

Table 3 Reaction time, yield, and melting points of newly synthesized compounds **6a–d**

Product	Structure[a]	Yield (%)[b]	Mp (°C)	Time (h)
6a		75–80	199–202	12
6b		70–75	210–211	12
6c		70–75	242–246	12
6d		65–70	257–258	12

[a]Reaction was carried out at reflux temperature in Ethanol.
[b]Isolated yields.

The structures of all the products were determined by detailed study of the spectroscopic data. All the synthesized compounds were characterized on the basis of spectral data and are evaluated for their antimicrobial activity. The structures of compounds **4a–k**, **5a–c**, and **6a–d** were established by ^{1}H NMR and mass spectral data. ^{1}H NMR of compound **4a** revealed the presence of multiplets at 7.14–7.98 δ for eight protons of the aromatic group, and a molecular ion peak exhibited at MS 305 (M+1) confirms the formation of compound **4a**. The conversion of compound **4a** to **5a** up on oxidation of **4a** with m-CPBA in DCM as solvent at room temperature directly confirmed LC-MS analysis. Thus the compound **5a** exhibited molecular ion peaks at MS 335 (M–1). The structure of compound **6a** was confirmed by its ^{1}H NMR spectra. It exhibited multiplets at 7.84–7.14 δ for four protons, which confirmed its aromatic protons, and a broad singlet at 10.5 δ for one proton revealed the presence of a NH group (D_2O exchangeable). Finally, in its mass spectra, it exhibited a molecular ion peak at MS 261 (M–1), which confirms its structure.

Antibacterial Activity

The activity was carried out using the cup plate agar diffusion method.[40] All the synthesized compounds (Table S1, available online in the Supplementary Materials) were tested for their antibacterial activity against various bacterial strains belonging to Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus haemolytius*) and Gram negative (*Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*). The zone of inhibition was measured in millimeters. Streptomycin was used as the standard drug for comparison. The compounds were tested at 40 μg/mL concentration, and DMSO was used as a vehicle. The results show that the synthesized compounds were found to be active against the tested bacteria. The maximum activity was found in **6a**, **6b**, **6c**, **6d**, and **5b** against all the bacteria, whereas **4a–e**, **5a**, and **5c** shows moderate activity.

Analgesic Activity[41]

Male *Swiss albino* mice were produced from Virus Diagnostic Laboratory, Shivamogga, Karnataka, India. Analgesic activity was calculated as the percentage maximum possible effect (% MPE), and the results are recorded in Table S2.

Analgesic properties of test compounds were compared with the activity of standard drug aspirin having percentage of protection value 62.1. The compounds **4a**, **4b** and **5b**, **5c** exhibited almost equipotent percentage protection as compared with standard aspirin and other compounds, and **5b**, **5c** and **6a**, 6**d** showed a more significant percentage of protection.

CONCLUSION

In conclusion, we have developed a simple methodology to prepare novel biologically important aryl/hetero aryl coumarinyl thioethers that resembles the structure of naturally occurring phenyl coumarinyl ethers. The antibacterial and analgesic activity results showed that the compounds coupled with nitrogen heterocycles are more active compared to sulfonyl- and thionyl-coupled compounds. Further testing must be carried out to check the minimum inhibitory concentration (MIC) values of the active compounds.

EXPERIMENTAL

The melting points are uncorrected. Column chromatography was performed on silica gel (60–120 mesh, Merck). Commercial reagents and solvents were of analytical grade or were purified by standard procedures prior to use. ^{1}H NMR was recorded on a Bruker Advance 400 MHz DPX spectrometer and referenced to TMS as internal standard. Mass spectra were run by electron impact at 70 eV in a VG Auto spectrometer. Selected NMR and mass spectra are shown in Figures S1–S6 (Supplemental Materials)

Synthesis of 3-Bromo-4-hydroxy-coumarin 3

4-Hydroxycoumarin (100 mg, 1 eq, 0.61 mmol) was dissolved in acetonitrile (10 mL), and powdered N-bromosuccinimide (1.1 eq, 0.67 mmol) was added at 25–27°C under constant stirring. The reaction mixture was poured into water. The precipitate thus obtained was filtered, dried, and purified by column chromatography using chloroform:methanol (9:1 v/v).

General Procedure for the Synthesis of 4-Hydroxy-3-(phenylthio)coumarins 4a–k

In a dry, round-bottom flask, a mixture of 3-bromo-4-hydroxy-coumarin (100 mg, 1 eq, 0.41 mmol), potassium carbonate (3 eq, 1.24 mmol), and thiophenol (3 eq, 1.39 mmol) was heated at 85°C for 5 h in DMF (10 mL) as solvent. The progress of the reaction was monitored by TLC, chloroform:methanol system (9:1 v/v). The reaction mixture was filtered, poured into water, and neutralized with 1.5 N HCl solution. The solid thus separated was filtered and washed with diethyl ether to remove excess thiophenols. Finally, the crude product was purified by column chromatography by using chloroform:methanol (9:1 v/v).

General Procedure for the Synthesis of 3-[(-Chlorophenyl)sulfonyl]-4-hydroxy-2H-chromen-ones 5a–c

3-[(3-Chlorophenyl)thio]-4-hydroxy-2*H*-chromen-2-one **4a** (100 mg, 0.328 mmol) was dissolved in dichloromethane (10 mL). To this, meta-chloroperbenzoic acid (2.5 eq, 0.82 mmol) was added. The reaction mixture was stirred at 25–27°C for about 8 h and was poured into 10% potassium carbonate solution to remove benzoic acid. Finally, the crude product was extracted with ethyl acetate, washed with brine solution, dried by anhydrous sodium sulfate, and purified by column chromatography using chloroform:methanol (9:1 v/v).

General Procedure for Synthesis of 4-Hydroxy-3-(imidazol/tetrazolo-2-yl)thio]-2h-chromen-2-ones 6a–d

In a dry, round-bottom flask, a mixture of **3** (100 mg, 1 eq, 0.41 mmol), cesium carbonate (Cs_2CO_3) (3 eq, 1.23 mmol), and mercapto compound (2 eq, 0.82 mmol) was heated at 70°C for 12 h in ethanol (10 mL) as solvent. The progress of the reaction was monitored by TLC, chloroform:methanol (9:1 v/v) system. The reaction mixture was filtered, poured into water, and neutralized with 1.5 N HCl solution. The solid obtained was filtered, and was washed with diethyl ether to remove the excess mercapto compound. Finally, the crude product was purified by column chromatography using chloroform:methanol (9:1 v/v).

Spectral Details

3-[(3-Chlorophenyl)thio]-4-hydroxy-2H-chro men-2-one (4a). Mp: 159–160°C; IR (cm^{-1}): 3422, 1719. NMR (DMSO-d_6): 7.98 (d, J = 8.0 Hz, 1H), 7.74 (t, J = 7.2 Hz, 1H), 7.44 (m, J = 8.0 Hz, 2H), 7.32 (t, J = 7.6 Hz, 1H), 7.22 (d, J = 7.2 Hz, 2H), 7.16 (d, J = 8.0Hz, 1H), m/z: 305 (M+1). Anal. Calcd. For $C_{15}H_9$ ClO_3S = C, 59.12; H, 2.98. Found: C, 59.05; H, 2.8.

3-[(3-Bromophenyl)thio]-4-hydroxy-2H-chromen-2-one (4b). Mp: 220–222°C; IR (cm^{-1}): 3418, 1719. NMR (DMSO-d_6): 7.97 (d, J = 7.6 Hz, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.67 (d, J = 8 Hz, 1H), 7.43 (m, J = 8 Hz, 2H), 7.24 (t, J = 7.6 Hz, 1H), 7.08 (t, J = 7.60 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), m/z: 347(M-1). Anal. Calcd. For $C_{15}H_9$ BrO_3S = C, 59.12; H, 2.98. Found: C, 58.95; H, 2.82.

3-[(3-Fluorophenyl)thio]-4-hydroxy-2H-chromen-2-one (4c). Mp: 166–167°C; IR (cm^{-1}): 3396, 1719. NMR (DMSO-d_6): 7.97 (d, J = 1.2 Hz, 1H), 7.72 (d, J = 0.8 Hz, 1H), 7.43 (t, J = 6.0 Hz, 2H), 7.29 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 5.6 Hz, 2H), 7.16 (d, J = 7.60 Hz, 1H, m/z: 287 (M-1). Anal. Calcd. For $C_{15}H_9$ FO_3S = C, 62.4; H, 3.15. Found: C, 61.7; H, 2.94.

4-Hydroxy-3-(phenylthio)-2H-chromen-2-one (4d). Mp: 200–202°C; IR (cm^{-1}): 3392, 1719. NMR (DMSO d_6): 7.97 (d, J = 6.8 Hz, 1H), 7.72 (t, J = 7.2 Hz, 1H), 7.44 (m, J = 5.2 Hz, 2H), 7.30 (t, J = 7.6 Hz, 2H), 7.19 (t, J = 7.6 Hz, 3H), m/z: 270 (M-1). Anal. Calcd. For $C_{15}H_{10}O_3S$ = C, 66.65; H, 3.73. Found: C, 66.52; H, 3.61.

3-[(4-Chlorophenyl)thio]-4-hydroxy-2H-chromen-2-one (4e). Mp: 197–198°C; IR (cm^{-1}): 3390, 1719. NMR (DMSO-d_6): 7.97 (d, J = 1.2 Hz, 1H), 7.73 (t, J = 7.2 Hz, 1H), 7.43 (t, J = 5.6 Hz, 2H), 7.33 (d, J = 7.2 Hz, 2H), 7.22 (d, J = 8.8Hz, 2H), m/z: 303 (M-1). Anal. Calcd. For $C_{15}H_9ClO_3S$ = C, 59.12; H, 2.98 Found: C, 58.89; H, 2.73.

4-Hydroxy-3-[(3-methoxyphenyl)thio]-2H-chromen-2-one (4f). Mp: 176–177°C; IR (cm^{-1}): 3389, 1719. NMR (DMSO-d_6): 7.97 (d, J = 8 Hz, 2H), 7.73 (t, J = 7.2 Hz, 3H), 7.44 (m, J = 5.6 Hz, 2H), 7.21 (t, J = 8.4 Hz, 1H), 6.74 (t, J = 7.2 Hz, 3H), 3.75 (s,3H), m/z: 299 (M-1). Anal. Calcd. For $C_{16}H_{12}O_4S$ = C, 63.99; H, 4.03 Found: C, 63.88; H, 3.9.

3-[(2-Bromophenyl)thio]-4-hydroxy-2H-chromen-2-one (4g). Mp: 210–213°C; IR (cm^{-1}): 3388. NMR (DMSO-d_6): 7.98 (d, J = 8 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.62 (d, J = 8 Hz, 1H), 7.45 (m, J = 8.4 Hz, 2 H), 7.23 (t, J = 7.20 Hz, 1H), 7.09 (d, J = 7.6 Hz, 1H), 6.92 (m, J = 0.8 Hz,1H), m/z: 347(M-1). Anal. Calcd. For $C_{15}H_9$ BrO_3S = C, 51.59; H, 2.6 Found: C, 51.52; H, 2.41.

3-[(2-Fluorophenyl)thio]-4-hydroxy-2H-chromen-2-one (4h). Mp: 220–222°C; IR (cm^{-1}): 3394, 1721. NMR (DMSO-d_6): 8.05 (d, J = 1.6 Hz, 1H), 7.74 (d, J = 8.1 Hz, 1H), 7.46 (m, J = 7.6, 2H), 7.22 (s, 1H), 7.15 (m, J = .4 Hz, 3H), m/z: 287 (M-1). Anal. Calcd. For $C_{15}H_9$ FO_3S = C, 62.49; H, 3.15 Found: C, 62.38; H, 3.02.

4-Hydroxy-3-([2-(trifluoromethyl) phenyl]thio)-2H-chromen-2-one (4i). Mp: 227–228°C; IR (cm^{-1}): 3417, 1719. NMR (DMSO-d_6): 7.94 (d, J = 7.9 Hz, 1H),7.72 (t, J = 8 Hz, 1H),7.58 (d, J = 7.6 Hz, 1H), 7.42 (m, J = 8 Hz, 2H), 7.23 (m, J = 8 Hz, 1H), 7.07 (m, J = 4 Hz, 1H), 6.90 (d, J = 8 Hz, 1H), m/z: 337(M-1). Anal. Calcd. For $C_{16}H_9$ F_3O_3S = C, 56.80; H, 2.68 Found: C, 56.68; H, 2.55.

4-Hydroxy-3-[(3-(trifluoromethyl)phenyl)thio]-2H-chromen-2-one (4j). Mp: 217–218°C; IR (cm^{-1}): 3420, 1723. NMR (DMSO-d_6): 7.97 (d, J = 6.8 Hz, 1H), 7.73

(t, J = 7.2 Hz, 1H), 7.5 (t, J = 4.0 Hz, 3H), 7.39 (m, J = 7.6 Hz) m/z 337 (M-1). Anal. Calcd. For $C_{16}H_9F_3O_3S$ = C, 56.80; H, 2.68 Found: C, 56.61; H, 2.53.

4-Hydroxy-3-[(4-(trifluoromethyl)phenyl)thio]-2H-chromen-2-one (4k). Mp: 136–137°C; IR (cm^{-1}) 3398, 1727. NMR (DMSO-d_6): 7.99 (d, J = 8.0 Hz, 1H), 7.74 (m, J = 1.2 Hz, 1H), 7.61 (d, J = 8.4 Hz, 2H), 7.44 (t, J = 8.4 Hz, 2H), 7.369(t, J = 8.0 Hz, 2H), m/z 337 (M-1). Anal. Calcd. For $C_{16}H_9F_3O_3S$ = C, 56.80; H, 2.68 Found: C, 56.62; H, 2.51.

3-[(3-Chlorophenyl)sulfonyl]-4-hydroxy-2H-chromen-2-one (5a). Mp: 170–172°C; IR (cm^{-1}): 3432, 1738, 1338–1288. NMR (DMSO-d_6): 7.98 (d, J = 8.0 Hz, 1H), 7.74 (t, J = 7.2 Hz, 1H), 7.44 (m, J = 8.0 Hz, 2H), 7.32 (t, J = 7.6 Hz, 1H), 7.22 (d, J = 7.2 Hz, 2H), 7.16 (d, J = 8.0 Hz, 1H), m/z 335 (M-1). Anal. Calcd. For $C_{15}H_9ClO_5S$). Anal. Calcd. For $C_{16}H_9F_3O_3S$ = C, 53.5; H, 2.69 Found: C, 52.8; H, 2.55.

3-[(3-Bromophenyl)sulfonyl]-4-hydroxy-2H-chromen-2-one (5b). Mp: 202–204°C; IR (cm^{-1}): 3438, 1731, 1341–1294. NMR (DMSO-d_6): 7.97 (d, J = 7.6 Hz, 1H), 7.73 (t, J = 7.6 Hz, 1H), 7.67 (d, J = 8 Hz, 1H), 7.43 (m, J = 8 Hz, 2H), 7.24 (t, J = 7.6 Hz, 1H), 7.08 (t, J = 7.60 Hz, 1H), 6.91 (d, J = 8.4 Hz,1H), m/z 379 (M-1). Anal. Calcd. For $C_{15}H_9BrO_5S$ = C, 47.26; H, 2.38 Found: C, 47.11; H, 2.17.

3-[(3-Fluorophenyl)sulfonyl]-4-hydroxy-2H-chromen-2-one (5c). Mp: 130–133°C; IR (cm^{-1}): 3428, 1727, 1329–1310. NMR (DMSO-d_6): 7.97 (d, J = 1.2 Hz, 1H), 7.72 (d, J = 0.8 Hz, 1H), 7.43 (t, J = 6.0 Hz, 2H), 7.29 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 5.6 Hz, 2H), 7.16 (d, J = 7.60 Hz, 1H, m/z 319 (M-1). Anal. Calcd. For $C_{15}H_9FO_5S$ = C, 56.25; H, 2.83 Found: C, 56.08; H, 2.72.

4-Hydroxy-3-(1H-imidazol-2-ylthio)-2H-chromen-2-one (6a). Mp: 75–80°C; ^{1}H NMR (DMSO d_6): 7.84 (d, J = 1.6 Hz, 1H), 7.42 (t, J = 6.4 Hz, 1H), 7.14 (m, J = 1.2 Hz, 2H), 10.5 (*b*, *s*, 1H, NH, D_2 O exchangable), 6.76 (*d*, 2H, = CH), *m/z:* 261 (M-1) (100%), 211(25%), 157 (48%), 133 (70%). Anal. Calcd. For $C_{12}H_8N_2O_3S$ = C, 55.38; H, 3.10; N, 10.76 Found: C, 55.29; H, 2.87; N, 10.54.

4-Hydroxy-3-[(1-methyl-1H-imidazol-2-yl)thio]-2H-chromen-2-one (6b). Mp: 70–75°C; ^{1}H NMR (DMSO d_6): 7.77 (d, J = 1.2 Hz, 1H), 7.39 (t, J = 1.6 Hz, 1H), 7.10 (m, J = 7.6 Hz, 2H), 3.64 (s, 3H), 6.69 (*d*, 2H, = CH), m/z: 275 (M-1). Anal. Calcd. For $C_{13}H_{10}N_2O_3S$ = C, 56.92; H, 3.67; N, 10.21 Found: C, 56.83; H, 3.52; N, 10.02.

4-Hydroxy-3-(1H-tetrazol-5-ylthio)-2H-chromen-2-one (6c). Mp: 70–75°C; ^{1}H NMR (DMSO d_6): 7.78 (d, J = 2 Hz, 1H), 7.45 (t, J = 1. 6 Hz, 1H), 7.15 (m, J = 7.2 Hz, 2H), m/z: 263 (M-1). Anal. Calcd. For $C_{10}H_6N_4O_3S$ = C, 45.8; H, 2.31; N, 21.36 Found: C, 45.78; H, 2.16; N, 21.19.

4-Hydroxy-3-[(1-methyl-1H-tetrazol-5-yl)thio]-2H-chromen-2-one (6d). Mp: 65–70°C; ^{1}H NMR (DMSO d_6): 7.78 (d, J = 2 Hz, 1H), 7.45 (t, J = 1. 6 Hz, 1H), 7.15 (m, J = 7.2 Hz, 2H), 3.9 (s,3H, -CH_3), m/z: 277 (M-1). Anal. Calcd. For $C_{11}H_8N_4O_3S$ = C, 47.82; H, 2.92; N, 20.28 Found: C, 47.68; H, 2.75; N, 20.07.

BIOLOGICAL ACTIVITY

Antibacterial Activity

The present investigation deals with the antibacterial activity of synthesized compounds which were studied comparatively with that of the antibiotic streptomycin, using cup-plate method against *Streptococcus haemolyticus*, *Staphylococcus aureus*, *Salmonella*

typhi, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, and *Klebsiella pneumonia.*

Analgesic Activity: Materials and Methods

Colony bred albino mice (Swiss strain) of either sex weighing 22–35 g were used to evaluate analgesic activity.[41] Analgesic activity was determined as described by the method based on acetic acid–induced writhing in mice. The percentage of protection of the compounds was compared with the activity of acetyl salicylic acid as standard drug, and was calculated by using the following formula:

$$\% \text{ of inhibition} = [1 - \text{Nt/Nc}] \times 100$$

where, Nt = mean number of writhing in test animals and Vc = mean number of writhing in control. The results of analgesic activities are described in Table S2.

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